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Abstract:	<p>Based on genetic, chemotaxonomic and phenotypic characteristics, a novel species belonging to the genus <i>Vibrio</i> is described. The facultative anaerobic strain S2757T was isolated from a mussel collected in the Solomon Sea (Solomon Islands). Phylogenetic analyses based on sequences of 16S rRNA and <i>fur</i> genes indicated the affiliation of the strain to a new species. This observation was supported by a multilocus sequence analysis (MLSA) including sequences of the housekeeping genes 16S rRNA, <i>gyrB</i>, <i>pyrH</i>, <i>recA</i> and <i>topA</i>. In silico DNA-DNA hybridization (DDH) and Average Nucleotide Identity (ANI) values comparing the genomic sequence of strain S2757T with those of closely related type strains were lower than 23 and 82 %, respectively. The DNA G+C content of the strain was 45.3 mol%. Phenotypic and chemotaxonomic analyses clearly differentiated the strain from other <i>Vibrio</i> species. Hence, strain S2757T should be considered a novel species in the genus <i>Vibrio</i>. The name <i>Vibrio galathea</i> sp. nov. is proposed, with S2757T (= DSM 100497T = LMG 28895T) as the type strain.</p>

***Vibrio galathea* sp. nov., a novel member of the *Vibrionaceae* family isolated from the Solomon Sea.**

Sonia Giubergia^{1,2}, Henrique Machado^{1,2}, Ramona Valentina Mateiu³ and Lone Gram^{1*}

¹NovoNordisk Foundation Centre for Biosustainability, Technical University of Denmark, Kogle Allé 6, DK-2970 Hørsholm

² Department of Systems Biology, Technical University of Denmark, Matematiktorvet bldg. 301, DK-2800 Kgs. Lyngby.

³ Center for Electron Nanoscopy, Technical University of Denmark, Fisikvej bldg. 307, DK-2800 Kgs. Lyngby.

*corresponding author: email: gram@bio.dtu.dk

Phone: +45 252586

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The GenBank accession number for the *fur* gene sequence of *Vibrio sinaloensis* DSM 21326^T is KT380049.

GenBank accession numbers for the whole genome sequences of *Vibrio hepatarius* DSM 19134^T, *Vibrio xuii* DSM 17185^T, and *Vibrio nereis* DSM 19584^T are LHPI01, LHPK01, LHPJ01, respectively. Accession numbers of all nucleotide sequences used in this work, including those previously publicly available, are listed in Table S1.

Based on genetic, chemotaxonomic and phenotypic characteristics, a novel species belonging to the genus *Vibrio* is described. The facultative anaerobic strain S2757^T was isolated from a mussel collected in the Solomon Sea (Solomon Islands). Phylogenetic analyses based on sequences of 16S rRNA and *fur* genes indicated the affiliation of the strain to a new species. This observation was supported by a multilocus sequence analysis (MLSA) including sequences of the housekeeping genes 16S rRNA, *gyrB*, *pyrH*, *recA* and *topA*. *In silico* DNA-DNA hybridization (DDH) and Average Nucleotide Identity (ANI) values comparing the genomic sequence of strain S2757^T with those of closely related type strains were lower than 23 and 82 %, respectively. The DNA G+C content of the strain was 45.3 mol%. Phenotypic and chemotaxonomic analyses clearly differentiated the strain from other *Vibrio* species. Hence, strain S2757^T should be considered a novel species in the genus *Vibrio*. The name *Vibrio galathea* sp. nov. is proposed, with S2757^T (= DSM 100497^T = LMG 28895^T) as the type strain.

Members of the *Vibrionaceae* family are Gram-negative bacteria widespread in aquatic environments (Thompson *et al.*, 2004). Vibrios have been isolated as both planktonic and surface-associated organisms from several ecosystems, including seawater, marine sediments and animals (Thompson *et al.*, 2004). The number of vibrios colonizing different environmental niches can vary over orders of magnitude, depending on factors such as availability of nutrients, temperature and salinity (Takemura *et al.*, 2014). For instance, *Vibrio* species were shown to account for more than 50% of the total microbiota during a bacterial bloom that was possibly due to an increase in the concentration of available nutrients (Gilbert *et al.*, 2012).

A number of vibrios have been intensively studied because of their role as pathogens (Ben-Haim *et al.*, 2003; Faruque *et al.*, 1998; Jones & Oliver, 2009; Ramamurthy *et al.*, 2014) and symbionts (Nyholm *et al.*, 2000). In recent years, *Vibrionaceae* have also emerged as a reservoir of secondary metabolites with therapeutic applications, including antibacterial, anticancer and antifungal activities (Månsson *et al.*, 2011). Here, we report the taxonomic characterization of a strain belonging to the genus *Vibrio*. Strain S2757^T was isolated in 2007

from a mussel collected in the Solomon Sea (Solomon Islands) during the Galathea 3 global research expedition (<http://www.galathea3.dk/uk>) and was affiliated to the *Vibrionaceae* family based on its 16S rRNA gene sequence, as previously described (Gram *et al.*, 2010).

The type strains included in this study *V. brasiliensis* DSM 17184^T (Thompson *et al.*, 2003a), *V. orientalis* DSM 19136^T (Yang *et al.*, 1983), *V. hepatarius* DSM 19134^T (Thompson *et al.*, 2003b), *V. tubiashii* DSM 19142^T (Hada *et al.*, 1984), *V. sinaloensis* DSM 21326^T (Gomez-Gil *et al.*, 2008), *V. xuii* DSM 17185^T (Thompson *et al.*, 2003a) and *V. nereis* DSM 19584^T (Baumann *et al.*, 1980) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). All strains were routinely cultivated on Marine Agar (MA) (212185, Difco) or in Marine Broth (MB) (279110, Difco) at 25 °C. Strain S2757^T grew as small (2-4 mm), round, beige colonies after 48 hours on MA at 25 °C.

Cell morphology of strain S2757^T was observed by means of phase-contrast microscopy (x 1,000 magnification in Olympus BX51) and Scanning Electron Microscopy (FEI Quanta 200 FEG ESEM) after growth in filtered MB for 24 hours at 25 °C. Gram testing and catalase activity were assessed with the 3 % KOH (Gregersen, 1978) and the 3 % H₂O₂ (Cowan, 1974) methods, respectively. Oxidase activity was determined on a BBLTM DrySlideTM Oxidase (231746, BD Diagnostics) following manufacturers' instructions. Test of susceptibility to the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropyl pteridine, 10 and 150 µg/disc) was performed on Iso-sensitest agar (CM04741B, Oxoid) supplemented with 1.5 % NaCl and incubation at 25 °C for 48 hours. Salinity requirements of strain S2757^T were determined in synthetic ZoBell medium (5 g/L Bacto peptone, 1 g/L yeast extract, 0.1 g/L ferric citrate) (ZoBell, 1941) with different NaCl concentrations (0 to 9 % w/v) at 28 °C. Growth was assessed using a microplate reader (Spectra Max i3, Molecular Devices). The temperature range for growth was determined on MA. The ability of strain S2757^T to grow in anaerobic conditions was tested on MA at 25 °C using an anaerobic jar and anaerobic atmosphere generation bags (68061, Fluka).

70

71 Physiological and biochemical characterization using API 20 NE strips (20050, Biomerieux), API ZYM strips
72 (25200, Biomerieux) and Biolog GN2 plates (Biolog Inc., USA) was done on strain S2757^T and on the closely
73 related species *V. tubiashii* DSM 19142^T, *V. brasiliensis* DSM 17184^T, *V. orientalis* DSM 19136^T and *V.*
74 *hepatarius* DSM 19134^T. Bacterial suspensions were prepared in 1.5 % w/v NaCl using biomass grown
75 overnight on MA at 25 °C. Inoculation of strips and plates was done in agreement with the manufacturers'
76 instructions. Cellular fatty acid of strain S2757^T and related species were analyzed as methyl esters (FAME) by
77 gas chromatography. The analysis was performed in duplicates by the DSMZ using biomass grown for 24 hours
78 on MA at 25 °C and according to the instructions of the Microbial Identification System (MIDI Inc., USA). Cell
79 morphology of strain S2757^T and related species was observed on thiosulfate-citrate-bile-sucrose (TCBS,
80 CM0333, Oxoid) agar plates. Detailed morphological, physiological and biochemical features distinguishing
81 strain S2757^T from related species are summarized in Table 1 and in the species description. The complete list of
82 the results of the performed tests and analyses is available in Table S2.

83

84 Cells of S2757^T were Gram negative, slightly curved rod shaped (1.5 ± 0.4 µm in length) and motile by means
85 of one polar flagellum (0.7 ± 0.2 µm in length) (Figure S1). Strain S2757^T was catalase and oxidase positive, and
86 sensitive to the vibriostatic agent O/129. NaCl was required for growth and tolerated up to a concentration of 8
87 % w/v. Strain S2757^T grew as green, small (2-3 mm) colonies on TCBS agar. Strain S2757^T produced α-
88 glucosidase but not acid phosphatase, N-acetyl-β-glucosaminidase and lipase. The strain could utilize D-glucose-
89 6-phosphate and D-alanine, but not L-threonine, L-proline and sucrose. The major cellular fatty acids of strain
90 S2757^T were summed feature 3 (16:1 ω7c and/or 16:1ω6c and/or 15 iso 2OH), 16:0, and summed feature 8 (18:1
91 ω7c and/or 18:1 ω6c). These values were comparable to those of the closely related species; however, the fatty
92 acid pattern of strain S2757^T was distinct due to the presence of a relatively high amount (9.8% in total) of the

93 fatty acids 15:0 iso, 16:0 iso and 17:0 iso compared to the patterns of the other analyzed species, for which
94 values were lower than 1.2 %.

95

96 For strains *V. hepatarius* DSM 19134^T, *V. xuii* DSM 17185^T and *V. nereis* DSM 19584^T no whole genome
97 sequence was publicly available at the time this study was started. Therefore, high purity genomic DNA was
98 obtained as described previously (Sambrook & Russel, 2001) by repeated phenol:chloroform:isoamyl alcohol
99 purification steps followed by RNase treatment and DNA precipitation. Quantification was performed on a
100 NanoDrop Spectrometer (Saveen Werner, Sweden) and a Qubit 2.0 Analyser (Invitrogen, United Kingdom).
101 Genome sequencing was carried out at the NovoNordisk Foundation Center for Biosustainability (Hørsholm,
102 Denmark). Libraries of 300-400 bp were prepared and used for 151 bp paired-end sequencing by Illumina
103 sequencing technology on a MiSeq sequencer. Data were assembled to contigs using the *de novo* assembly
104 algorithm of CLC Genomic Workbench, version 7 (CLC Bio, Aarhus, Denmark). The list of the GenBank/EBI
105 accession numbers of the nucleotide sequences used in this study, including those herein generated, is available
106 in Table S1. For the *in silico* phylogenetic analysis, sequences of the single genes were obtained directly from
107 the GenBank database or extracted from whole genome sequences based on their PGAP (NCBI Prokaryotic
108 Genome Annotation Pipeline) annotation (Tatusova *et al.*, 2013) or by BLAST search using CLC Main
109 Workbench Version 7.6.2 (CLC Bio, Aarhus, Denmark).

110 The comparison of the 1487 bp long 16S rRNA gene sequence obtained from the complete genome sequence of
111 the new isolate with those from type strains available in the GenBank database using the BLASTN algorithm
112 (<https://blast.ncbi.nlm.nih.gov>) and the Ez-Taxon-e service (<http://www.ezbiocloud.net/eztaxon>) confirmed that
113 strain S2757^T belongs to the genus *Vibrio*, as previously established (Gram *et al.*, 2010). Pairwise alignment of
114 the almost complete 16S rRNA gene sequences was carried out using CLC Main Workbench. A phylogenetic
115 tree was constructed in MEGA6 (Tamura *et al.*, 2013) using the Neighbor-Joining method. The robustness of the
116 tree topology was tested with 1000 bootstrap iterations (Fig.1). Based on the 16S rRNA gene sequences, strain

117 S2757^T was phylogenetically closely related to *V. hepatarius* DSM 19134^T, *V. brasiliensis* DSM 17184^T, *V.*
118 *maritimus* R 40493^T and *V. tubiashii* DSM 19142^T sharing 98.5%, 98.3%, 98.2% and 97.8% 16S rRNA gene
119 sequence similarity, respectively. However, due to the low interspecies resolution which can be obtained in
120 *Vibrionaceae* by using the 16S rRNA gene sequence (Sawabe *et al.*, 2007), two phylogenetic trees based on
121 complete sequences of the recently proposed *Vibrionaceae* phylogenetic marker *fur* gene (Machado & Gram,
122 2015) (Fig. 2) and on the concatenated sequences of five housekeeping genes (Fig. 3) were constructed. These
123 phylogenetic trees were obtained as described above and elsewhere (Machado & Gram, 2015; Sawabe *et al.*,
124 2013; Thompson *et al.*, 2005). For the *fur* gene phylogenetic tree, gene sequences were obtained either by PCR
125 based gene amplification followed by sequencing as described previously (Machado & Gram, 2015), or from
126 whole genome sequences as described above. For the multilocus sequence analysis (MLSA), sequences of the
127 16S rRNA, DNA gyrase subunit B (*gyrB*), uridylate kinase (*pyrH*), recombinant protein RecA (*recA*) and DNA
128 topoisomerase I (*topA*) genes were retrieved from the GenBank database or from whole genome sequences, as
129 described above. Sequences were trimmed to a common length and concatenated to a final length of 3800 bp.
130 Both phylogenetic trees showed that strain S2757^T was clearly separated from the other analyzed *Vibrio* species.
131 Whole genomes sequences of strain S2757^T and closely related species were compared by DNA-DNA
132 Hybridization (DDH) and Average Nucleotide Identity (ANI) values obtained *in silico* using the Genome-to-
133 Genome Distance calculator 2.0 (GGDC) provided by the DSMZ (<http://ggdc.dsmz.de/>) (Meier-Kolthoff *et al.*,
134 2013) and the Average Nucleotide Identity calculator (<http://enve-omics.ce.gatech.edu/ani/>) developed by the
135 Kostas Lab (Goris *et al.*, 2007). All DDH and ANI values were below the thresholds used for species definition
136 (70 % for DDH and 95 % for ANI) and identified *V. tubiashii* ATCC 19109^T as the closest relative of strain
137 S2757^T, with DDH = 22.50 % and ANI = 81.13% (Table 2). The G + C content of S2757^T calculated *in silico*
138 using CLC Main Workbench was 45.3 mol%, which is in agreement with values reported in literature for *Vibrio*
139 species.

140 The presented results indicate that strain S2757^T should be classified as a novel species in the genus *Vibrio*, for
141 which the name *Vibrio galathea* sp. nov. is proposed.

142

143 **Description of *Vibrio galathea* sp.nov.**

144 *Vibrio galathea* (ga.la.the'ae. N.L. gen. n. galathea, referring to the name of the Danish research expedition
145 Galathea 3 during which the type strain was isolated).

146 Cells are slightly curved rods, Gram-negative and motile by means of one polar flagellum. Colonies are circular,
147 beige in color and 2–4 mm in size after 48 hours at 25 °C on MA and round, green and 2–4 mm in size after 48
148 hours at 25 °C on TCBS. Growth occurs in presence of 0.5–8 % (w/v) NaCl in synthetic ZoBell medium, with
149 optimal growth at 2–5 %. The strain grows at 15–40 °C, with optimal growth at 25–30 °C. Growth is observed
150 under anaerobic conditions. The strain is positive for catalase and oxidase and sensitive to the vibriostatic agent
151 O/129. Strain S2757^T reduces nitrates to nitrites, produces indole and hydrolyzes esculin. Positive for alkaline
152 phosphatase, esterase lipase, leucine arylamidase, valine arylamidase and cysteine arylamidase but not for lipase
153 and acid phosphatase. Strain S2757^T can utilize as sole carbon sources: N-acetyl-D-glucosamine, D-cellobiose,
154 D-fructose, α -D-glucose, maltose, D-mannitol, D-mannose, D-trehalose, D-gluconic acid, D,L-lactic acid, D-
155 alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid,
156 inosine, uridine thymidine, α -D-glucose-1-phosphate and D-glucose-6-phosphate. It cannot utilize: N-acetyl-D-
157 galactosamine, sucrose, succinic acid, glycyl-L-aspartic acid, L-proline, L-threonine and glycerol. The most
158 abundant fatty acids of strain S2757^T are summed feature 3 (comprising 16:1 ω 7c and/or 16:1 ω 6c and/or 15 iso
159 2OH), 16:0, and summed feature 8 (comprising 18:1 ω 7c and/or 18:1 ω 6c).

160 The type strain, S2757^T (= DSM 100497^T = LMG 28895^T), was isolated from a mussel collected in the Solomon
161 Sea, Solomon Islands. The DNA G+C content of the type strain is 45.3 mol%.

162

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167 auspices of the Danish Expedition Foundation. This is Galathea 3 contribution no. P114 (to be added if/when
168 accepted).

169

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238

239 **List of figures and tables**

240

241 **Figure 1.** Phylogenetic tree based on partial 16S rRNA gene sequences, obtained using the Neighbor-Joining
242 method. Numbers at nodes indicate the level of bootstrap based on 1000 replicates; only values >50 % are
243 shown. *Photobacterium aquae* was used as outgroup. Bar, 0.5 % estimated sequence divergence.

244

245 **Figure 2.** Phylogenetic tree based on complete *fur* gene sequences, obtained using the Neighbor-Joining method.
246 Numbers at nodes indicate the level of bootstrap based on 1000 replicates; only values >50 % are shown.
247 *Photobacterium aquae* was used as outgroup. Bar, 5 % estimated sequence divergence.

248

249 **Figure 3.** Phylogenetic tree based on concatenated sequences of five genes (16S rRNA, *gyrB*, *pyrH*, *recA* and
250 *topA*; approximately 3800 bp) obtained using the Neighbor-Joining method. The sizes of the gene sequences
251 were: 16S RNA, 1439 bp; *gyrB*, 738 bp; *pyrH*, 530 bp; *recA*, 554 bp and *topA*, 552 bp. Numbers at nodes
252 indicate the level of bootstrap based on 1000 replicates; only values >50 % are shown. *Photobacterium aquae*
253 was used as outgroup. Bar, 2 % estimated sequence divergence.

254

255 **Table 1.** Features differentiating strain S2757^T from closely related *Vibrio* species.

256 Species are identified as: (1) *Vibrio galathea* sp. nov., (2) *V. brasiliensis* DSM 17184^T, (3) *V. orientalis* DSM
257 19136^T, (4) *V. hepatarius* DSM 19134^T, and (5) *V. tubiashii* DSM 19142^T. G, green; Y, yellow; +, positive; -,
258 negative. All data were generated in this work in biological duplicates.

259

260 **Table 2.** Comparison of the genomic sequences of S2757^T and related species based on DNA-DNA
261 Hybridization (DDH) and two-way Average Nucleotide Identity (ANI) values obtained with *in silico* methods.
262 Taxa: (1) *V. galathea*; (2) *V. hepatarius* DSM 19134^T; (3) *V. xuii* DSM 17185^T; (4) *V. nereis* DSM 19584^T; (5)

263 *V. brasiliensis* LMG 20546^T = DSM 17184^T; (6) *V. orientalis* DSM 19136^T; (7) *V. tubiashii* ATCC 19109^T =
264 DSM 19142^T.

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Table 1. Features differentiating strain S2757^T from closely related *Vibrio* species.

Species are identified as: (1) *Vibrio galathea* sp. nov., (2) *V. brasiliensis* DSM 17184^T, (3) *V. orientalis* DSM 19136^T, (4) *V. hepatarius* DSM 19134^T, and (5) *V. tubiashii* DSM 19142^T. G, green; Y, yellow; +, positive; -, negative. All data were generated in this work in biological duplicates.

Characteristic	1	2	3	4	5
Citrate‡	+	+	-	-	-
Malic acid‡	+	+	-	-	+
Growth in/on:					
8 % NaCl	+	-	+	-	-
TCBS (colour)	G	Y	Y	Y	Y
Production of:					
Lipase†	-	+	+	+	+
α-glucosidase†	+	-	-	-	+
Acid phosphatase†	-	+	+	-	-
N-acetyl-β-glucosaminidase†	-	-	+	+	+
Utilization of:					
D-glucose-6-phosphate	+	+	+	-	+
L-threonine	-	+	-	+	-
L-proline	-	+	+	-	-
D-alanine	+	+	-	-	-
Sucrose	-	+	-	+	+
FAME:					
16:0	19.5 ± 0.2	24.8 ± 0.3	23.3 ± 0.4	22.6 ± 0.2	22.2 ± 0.2
15:0 iso	2.1	0.3	0.2	0.1	0.1
16:0 iso	3.7	0.5	0.6	1.1	0.4
17:0 iso	4.0	0.3	-	0.1	0.2
Summed feature 3*	34.7 ± 0.4	35.9 ± 0.4	44.7 ± 0.4	39 ± 0.4	35.8 ± 0.3
Summed feature 8*	15.8 ± 0.2	19.5 ± 0.2	10.6 ± 0.2	18.3 ± 0.2	23.0 ± 0.2

271

272 † API ZYM

273 ‡ API 20 NE

274 * Summed feature 3: one or more of 16:1 ω7c, 16:1 ω6c and/or 15:0 iso 2OH. Summed feature 8: 18:1 ω7c
275 and/or 18:1 ω6c.

276 **Table 2.** Comparison of the genomic sequences of S2757^T and related species based on DNA-DNA
 277 Hybridization (DDH) and two-way Average Nucleotide Identity (ANI) values obtained with *in silico* methods.
 278 Taxa: (1) *V. galathea*; (2) *V. hepatarius* DSM 19134^T; (3) *V. xuii* DSM 17185^T; (4) *V. nereis* DSM 19584^T; (5)
 279 *V. brasiliensis* LMG 20546^T = DSM 17184^T; (6) *V. orientalis* DSM 19136^T; (7) *V. tubiashii* ATCC 19109^T =
 280 DSM 19142^T.

		ANI ± SD (%)						
		1	2	3	4	5	6	7
DDH ± SD (%)	1		80.22 ± 6.17	80.51 ± 5.90	79.81 ± 5.89	80.32 ± 5.73	80.56 ± 5.81	81.13 ± 5.48
	2	20.00 ± 2.31		80.35 ± 5.76	82.56 ± 6.48	80.45 ± 6.00	80.24 ± 5.79	80.53 ± 6.16
	3	20.80 ± 2.33	20.90 ± 2.33		80.41 ± 6.06	81.29 ± 6.06	88.74 ± 4.55	81.61 ± 6.13
	4	19.70 ± 2.30	22.30 ± 2.36	35.50 ± 2.48		80.06 ± 5.91	80.06 ± 5.72	80.26 ± 5.90
	5	20.60 ± 2.32	20.30 ± 2.32	21.20 ± 2.34	19.90 ± 2.30		81.28 ± 6.01	81.14 ± 5.90
	6	20.70 ± 2.33	20.30 ± 2.31	38.00 ± 2.49	20.30 ± 2.32	21.40 ± 2.34		81.83 ± 6.99
	7	22.50 ± 2.36	20.60 ± 2.32	21.80 ± 2.35	20.30 ± 2.31	21.60 ± 2.34	21.80 ± 2.35	

Figure 1

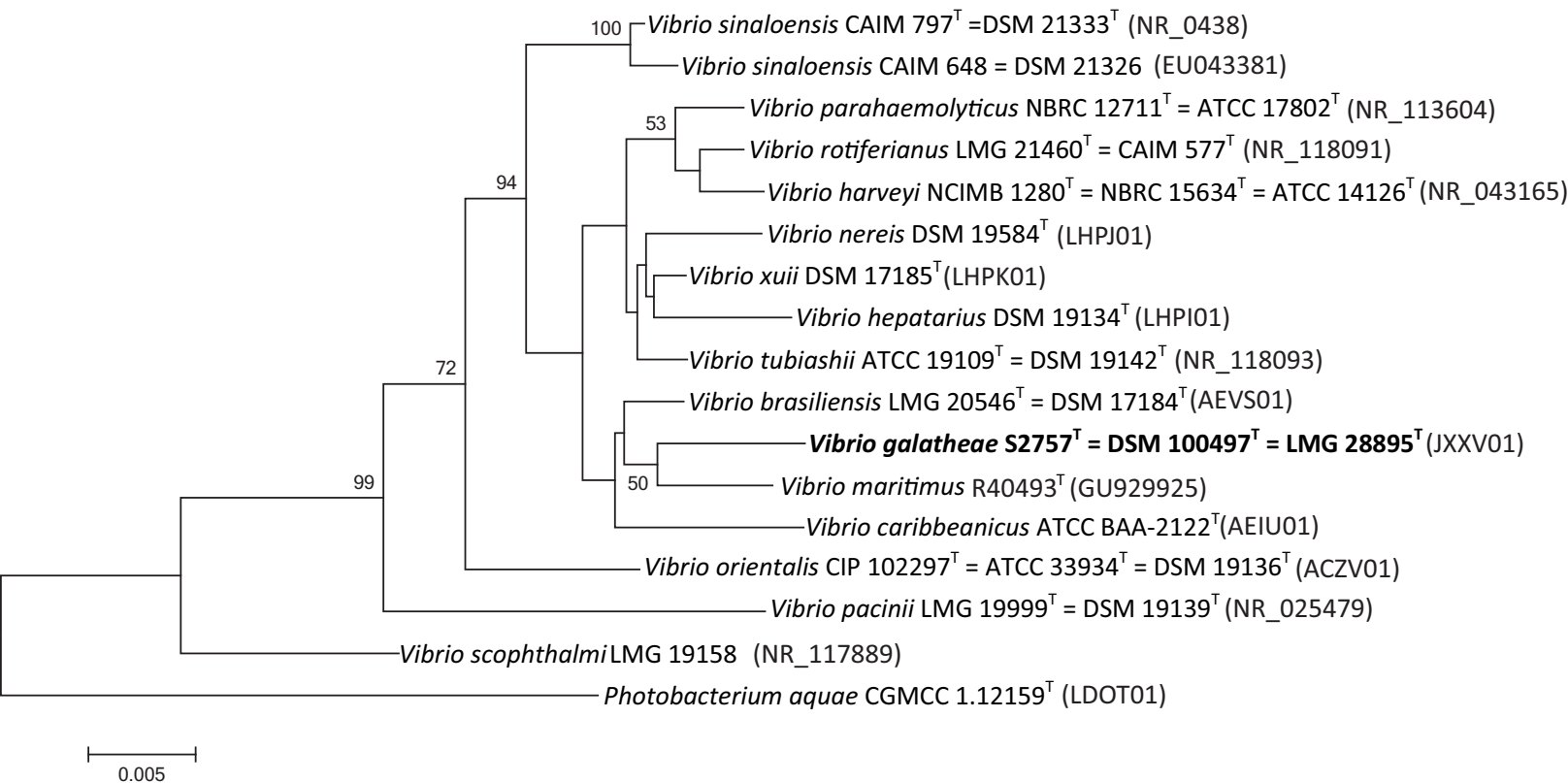


Figure 2

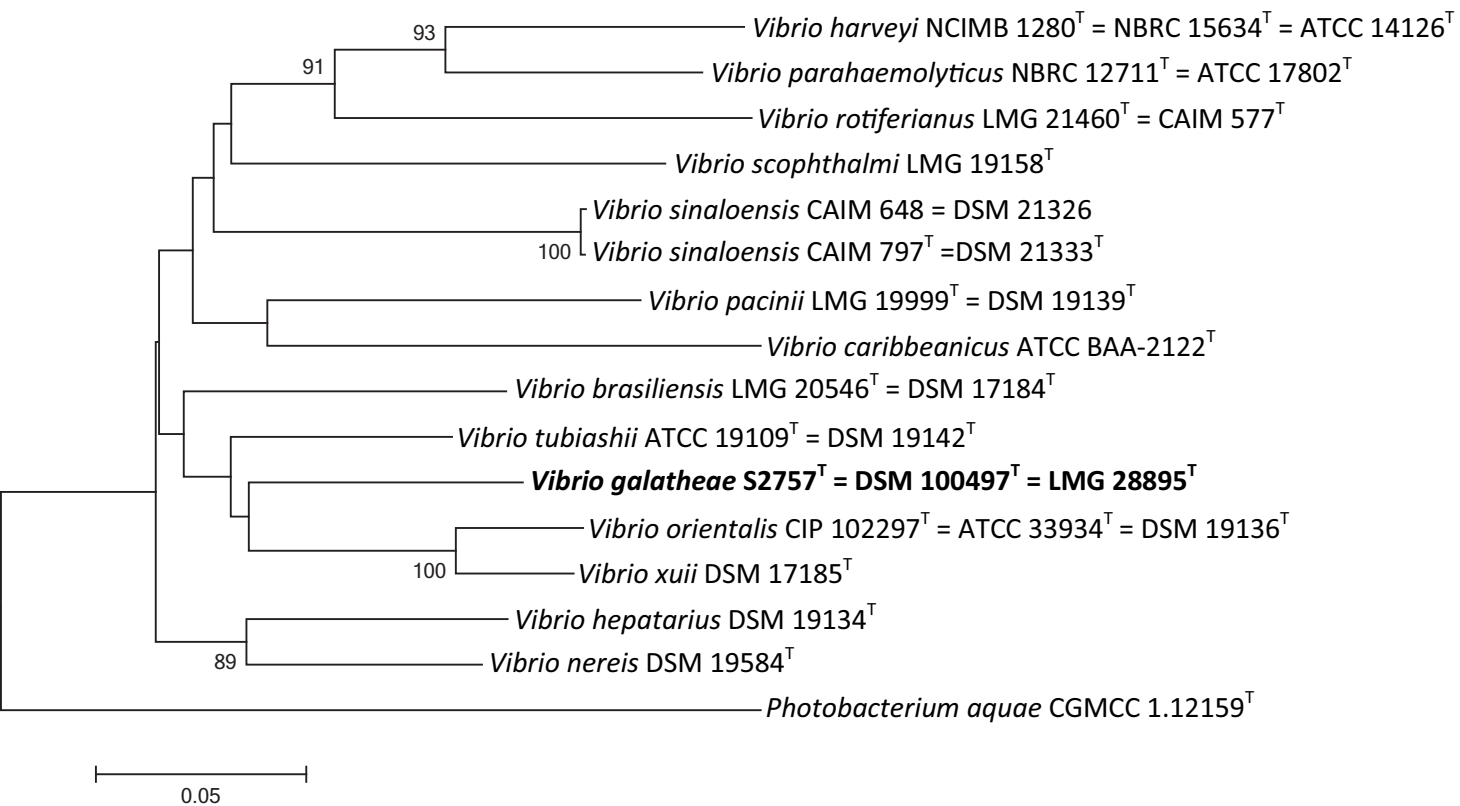
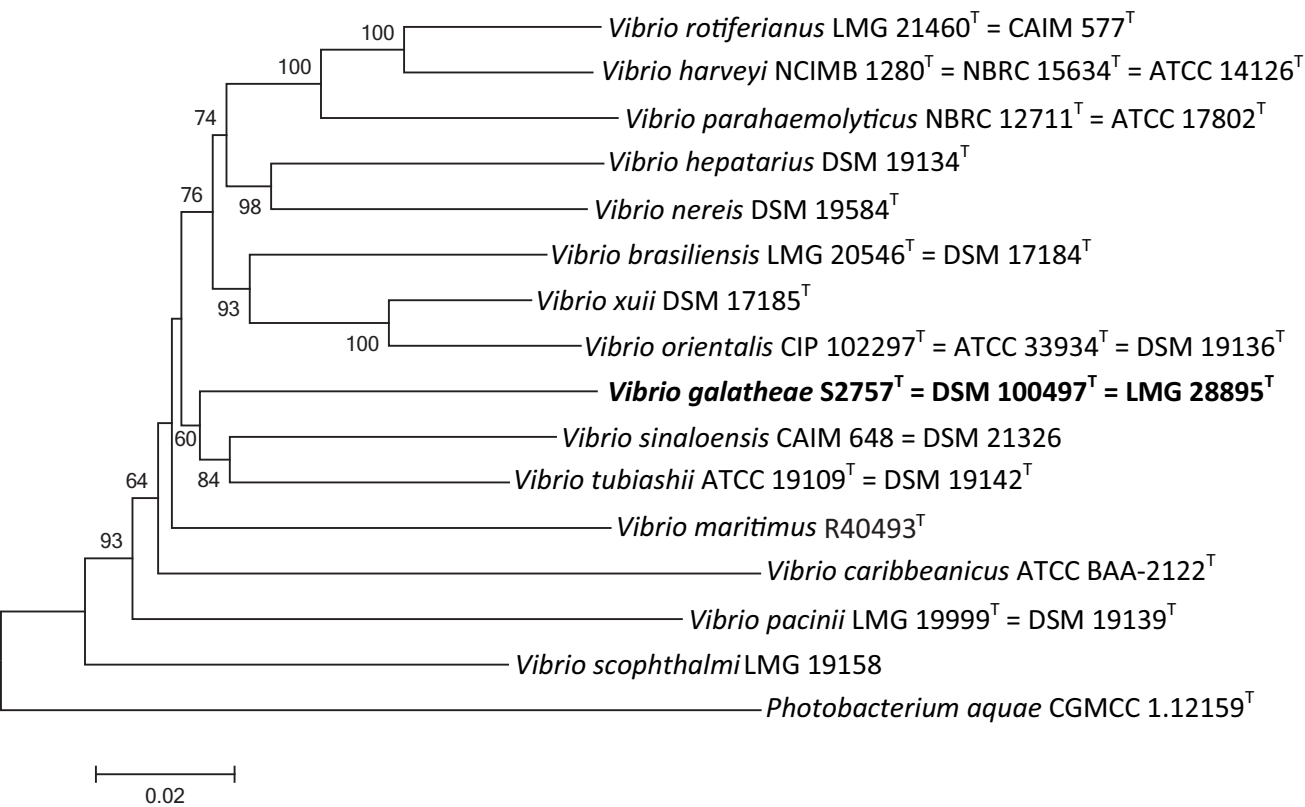
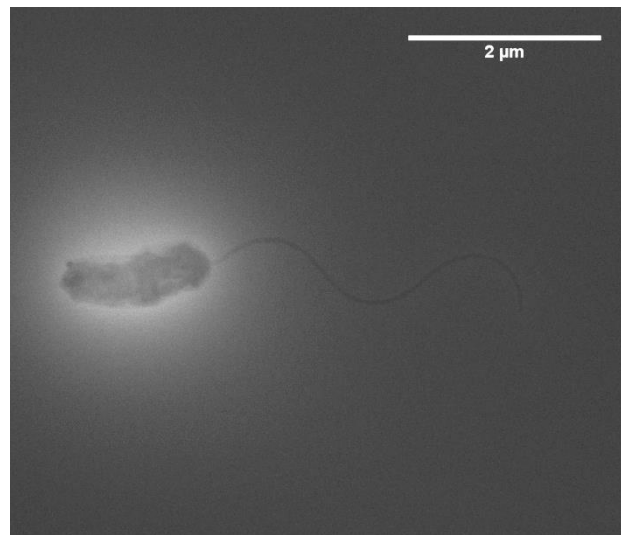


Figure 3





1
2 **Figure S1.** Wet scanning transmission electron Micrograph in Scanning Electron Microscope (wet-STEM SEM)
3 image of uranyl acetate stained strain S2757^T grown in MB for 24 hours at 25 °C.

4 **Table S1.** GenBank/EBI Accession numbers of the nucleotide sequences used in this study. For gene sequences that were extracted from
5 whole genome sequences, the WGS accession number is listed.

Species	Strain	GenBank/EBI accession number						
		WGS	16S rRNA	fur	gyrB	pyrH	recA	topA
<i>V. galathea</i>	S2757 ^T = DSM 100497 ^T = LMG 28895 ^T	JXXV01	JXXV01	JXXV01	JXXV01	JXXV01	JXXV01	JXXV01
<i>V. brasiliensis</i>	LMG 20546 ^T = DSM 17184 ^T	AEVS01	AEVS01	AEVS01	AEVS01	AEVS01	AEVS01	AEVS01
<i>V. orientalis</i>	CIP 102297 ^T = ATCC 33934 ^T = DSM 19136 ^T	ACZV01	ACZV01	ACZV01	ACZV01	ACZV01	ACZV01	ACZV01
<i>V. hepatarius</i>	DSM 19134 ^T	LHPI01*	LHPI01*	LHPI01*	LHPI01*	LHPI01*	LHPI01*	LHPI01*
<i>V. tubiashii</i>	ATCC 19109 ^T = DSM 19142 ^T	AFWI01	NR_118093	AFWI01	AFWI01	AFWI01	AFWI01	AFWI01
<i>V. caribbeanicus</i>	ATCC BAA-2122 ^T	AEIU01	AEIU01	AEIU01	AEIU01	AEIU01	AEIU01	AEIU01
<i>V. xuii</i>	DSM 17185 ^T	LHPK01*	LHPK01*	LHPK01*	LHPK01*	LHPK01*	LHPK01*	LHPK01*
<i>V. nereis</i>	DSM 19584 ^T	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*	LHPJ01*
<i>V. harveyi</i>	NCIMB 1280 ^T = NBRC 15634 ^T = ATCC 14126 ^T	BAOD01	NR_043165	BAOD01	BAOD01	BAOD01	BAOD01	BAOD01
<i>V. pacinii</i>	LMG 19999 ^T = DSM 19139 ^T	JONH01	NR_025479	JONH01	JONH01	JONH01	JONH01	JONH01
<i>V. parahaemolyticus</i>	NBRC 12711 ^T = ATCC 17802 ^T	LATW01	NR_113604	LATW01	LATW01	LATW01	LATW01	LATW01
<i>V. rotiferianus</i>	LMG 21460 ^T = CAIM 577 ^T	BAOI01	NR_118091	BAOI01	BAOI01	BAOI01	BAOI01	BAOI01
<i>V. scopthalmi</i>	LMG 19158 ^T	AFWE01	NR_117889	AFWE01	AFWE01	AFWE01	AFWE01	AFWE01
<i>V. sinaloensis</i>	CAIM 797 ^T =DSM 21333 ^T		NR_043858	KT380049*				
<i>V. sinaloensis</i>	CAIM 648 = DSM 21326	AEVT01	EU043381	AEVT01	AEVT01	AEVT01	AEVT01	AEVT01
<i>V. maritimus</i>	R 40493 ^T		GU929925		GU929929	GU929933	GU929935	GU929939
<i>P. aquae</i>	CGMCC 1.12159 ^T	LDOT01	LDOT01	LDOT01	LDOT01	LDOT01	LDOT01	LDOT01

* Sequences generated in this study.

8 **Table S2:** Results from the phenotypic and chemotaxonomic analyses. +, positive; -, negative, ND, not
9 determined; w, weak reaction.

Characteristic	Strains				
	<i>V. galathea</i>	<i>V. brasiliensis</i>	<i>V. orientalis</i>	<i>V. hepatarius</i>	<i>V. tubiashii</i>
water	-	-	-	-	-
a-cyclodextrin	-	-	-	-	-
dextrin	+	+	+	+	+
glycogen	+	+	+	+	+
Tween 40	w	+	+	w	+
Tween 80	w	+	+	w	+
N-acetyl-D-galactosamine	-	+	-	-	-
N-acetyl-D-glucosamine	+	+	+	+	+
adonitol	-	-	-	-	-
L-arabinose	-	+	-	-	-
D-arabitol	-	-	-	-	-
D-cellobiose	+	+	-	+	+
i-erythritol	-	-	-	+	-
D-fructose	+	+	-	+	+
L-fucose	-	+	-	-	-
D-galactose	-	-	-	w	-
Gentibiose	-	-	-	-	-
a-D-glucose	+	+	+	+	+
m-inositol	-	-	-	-	-
a-D-lactose	-	-	-	-	-
lactulose	-	-	-	-	-
maltose	+	+	+	+	+
D-mannitol	+	+	+	+	+
D-mannose	+	+	-	+	+
D-melibiose	-	-	-	-	-
b-methyl-D-glucoside	w	-	-	-	-
D-psicose	w	+	-	+	+
D-raffinose	-	-	-	-	-
L-rhamnose	-	-	-	-	-
D-sorbitol	-	+	-	-	-
sucrose	-	+	-	+	+
D-trehalose	+	+	+	+	+
turanose	-	-	-	-	-
xylitol	-	-	-	-	-
pyruvic acid methyl ester	w	+	-	w	-
succinic acid mono-methyl ester	-	+	-	-	-
acetic acid	-	+	-	-	w
cis-aconitic acid	-	-	-	-	-
citric acid	-	-	-	-	-
formic acid	-	-	-	-	-
D-galactonic acid lactone	-	-	-	-	-
D-galacturonic acid	-	-	-	-	-
D-gluconic acid	+	+	-	+	+
D-glucosaminic acid	-	-	-	-	-
D-glucuronic acid	-	-	-	-	+
a-hydroxybutyric acid	-	-	-	-	-
b-hydroxybutyric acid	-	+	-	-	w
g-hydroxybutyric acid	-	-	-	-	-
p-hydroxyphenylacetic acid	-	-	-	-	-
itaconic acid	-	-	-	-	-
a-ketobutyric acid	-	-	-	-	-

	Characteristic	Strains				
		<i>V. galathea</i>	<i>V. brasiliensis</i>	<i>V. orientalis</i>	<i>V. hepaticus</i>	<i>V. tubiashii</i>
	a-ketoglutaric acid	-	+	-	-	-
	a-ketovaleric acid	-	-	-	-	-
	D,L-lactic acid	+	+	-	+	+
	malonic acid	-	-	-	-	-
	propionic acid	-	-	-	-	-
	quinic acid	-	-	-	-	-
	D-saccharic acid	-	-	-	-	-
	sebacic acid	-	-	-	-	-
	succinic acid	-	+	+	+	+
	bromosuccinic acid	-	+	-	-	-
	succinamic acid	-	-	-	-	-
	glucuronamide	-	-	-	-	w
	L-alaninamide	-	-	-	-	-
	D-alanine	+	+	-	-	-
	L-alanine	+	+	+	-	+
	L-alanyl-glycine	+	+	+	+	+
	L-asparagine	+	+	+	+	+
	L-aspartic acid	+	+	+	+	+
	L-glutamic acid	+	+	+	+	+
	glycyl-L-aspartic acid	-	+	-	+	+
	glycyl-L-glutamic acid	+	+	-	+	+
	L-histidine	-	-	-	-	-
	hydroxy-L-proline	-	-	-	-	-
	L-leucine	-	-	-	-	-
	L-ornithine	-	+	-	-	-
	L-phenylalanine	-	-	-	-	-
	L-proline	-	+	+	-	-
	L-pyroglutamic acid	-	-	+	-	-
	D-serine	w	-	-	-	-
	L-serine	w	+	-	+	+
	L-threonine	-	+	-	+	-
	D,L-carnitine	-	-	-	-	-
	g-aminobutyric acid	-	-	-	-	-
	urocanic acid	-	-	-	-	-
	inosine	+	+	+	+	+
	uridine	+	+	+	+	+
	thymidine	+	+	+	+	+
	phenylethylamine	-	-	-	-	-
	putrescine	-	-	-	-	-
	2-aminoethanol	-	-	-	-	-
	2,3-butanediol	-	-	-	-	-
	glycerol	-	+	+	+	+
	D,L,a-glycerol phosphate	-	+	+	-	-
	a-D-glucose-1-phosphate	+	-	-	-	-
	D-glucose-6-phosphate	+	+	+	-	+
API ZYM	alkaline phosphatase	+	+	+	+	+
	esterase	+	+	+	+	+
	esterase lipase	+	+	+	+	+
	lipase	-	+	+	+	+
	leucine arylamidase	+	+	+	+	+
	valine arylamidase	+	+	-	+	-
	cystine arylamidase	+	+	-	+	-
	trypsin	-	-	-	-	-
	a-chymotrypsin	-	-	-	-	-
	acid phosphatase	-	+	+	-	-
	naphtol-AS-BI-phosphohydrolase	+	+	+	+	+
	a-galactosidase	-	-	-	-	-

Characteristic	Strains				
	<i>V. galathea</i>	<i>V. brasiliensis</i>	<i>V. orientalis</i>	<i>V. hepatarius</i>	<i>V. tubiashii</i>
b-galactosidase	-	-	-	-	-
b-glucuronidase	-	-	-	-	-
a-glucosidase	+	-	-	-	+
b-glucosidase	-	-	-	-	-
N-acetyl-b-glucosaminidase	-	-	+	+	+
a-mannosidase	-	-	+	-	-
a-fucosidase	-	-	-	-	-
Sum in Feature 2 12:0 aldehyde					
10:0 3OH		0,06			0,06
unknown 11.799					
12:0 iso	0,1				
12:0	3,4	3,2	3,6	3,6	3,4
unknown 12,484	0,4		0,5	0,3	
13:0 ISO	0,7	0,3	0,1	0,1	0,2
13:1 at 12-13		0,3		0,2	0,2
13:0		0,1	0,2	0,1	
13:0 iso 3 OH	0,3				
12:0 2OH	0,1	0,1	0,1	0,1	0,0
12:0 3OH	1,8	2,0	1,9	2,1	2,6
14:0 ISO	0,4	0,1	0,2	0,1	
14:0	6,5	6,4	9,1	6,3	6,5
unknown 14,502		0,1			
14:1 w5c			0,1		
15:0 ISO	2,1	0,3	0,2	0,1	0,1
15:0 ANTEISO	0,3	0,1	0,1	0,1	0,1
15:0	0,4	0,3	0,7	0,4	0,3
15:1 w8c					0,2
15:0 iso 3 OH	0,3				
14:0 ISO 3 OH	0,4	0,1		0,2	
Sum in feature 2 14:0 3OH/16:1 ISO I					
16:0 ISO	3,7	0,5	0,6	1,1	0,4
16:1 w9c		0,5	0,5	0,5	
16:1 w7c alcohol	0,6		0,3		
Sum in feature 3 16:1 w7c/16:1w6c					
Sum in feature 3 16:1 w7c/15 iso 2OH					
sum in feature 3 15:0 ISO 2OH/16:1w7c					
sum in feature 3 16:1 w6c/16:1 w7c					
16:1 w5c	0,2	0,2	0,3	0,3	0,3
16:0	19,5	24,8	23,3	22,6	22,2
17:0 ISO	4,0	0,3		0,1	0,2
17:0 anteiso	0,6			0,1	0,1
17:1 w8c	0,3	0,2	0,2	0,2	0,2
17:1 w9c	0,3				
17:0	0,4	0,2	0,4	0,3	0,3
16:0 3OH		0,3		0,3	0,4
Sum in feature 8 18:1 w7c	15,9			18,5	
Sum in feature 8 18:1 w6c					
18:1 w9c		0,3		0,3	0,3
18:1 w7c 11-methyl					0,2
18:1 w7c		19,5			
18:1 w5c				0,1	
18:0	0,3	0,4	0,3	0,4	0,4
18:0 iso	0,4				
sum in feature 7 un 18.846/19:1 w6c		0,4			
20:1 w7c				0,2	
Summed feature 2 *	2,1	2,6	2,0	2,2	2,4
Summed feature 3 *	34,7	35,9	44,7	39,0	35,8

FAME analysis

Characteristic	Strains				
	<i>V. galathea</i>	<i>V. brasiliensis</i>	<i>V. orientalis</i>	<i>V. hepaticus</i>	<i>V. tubiashii</i>
Summed feature 7 *		0,4		0,4	
Summed feature 8 *	15,8		10,6	18,3	23,0
Nitrates--> nitrites	+	+	+	+	+
L-tryptophane	+	+	+	+	+
D-glucose	-	+	-	-	+
L-arginine	-	-	-	-	-
urea	-	-	-	-	-
esculin ferric citrate (b-glucosidase)	+	+	-	-	-
gelatin (protease)	-	-	-	+	-
b-galactosidase	+	+	-	ND	+
D-glucose	-	-	-	-	-
L-arabinose	-	-	-	-	-
D-mannose	-	-	-	-	-
D-mannitol	-	-	-	-	-
N-acetylglucosamine	-	-	-	-	-
D-maltose	-	-	-	-	-
potassium gluconate	-	-	-	-	-
capric acid	-	-	-	-	-
adipic acid	-	-	-	-	-
malic acid	+	+	-	-	+
trisodium citrate	-	-	-	-	-
phenylacetic acid	-	-	-	-	-
oxidase	-	-	-	-	-

API 20 NE

*Summed feature 2: one or more among 12:0 aldehyde, 14:0 3OH and/or 16:1 iso; summed feature 3: one or more among 16:1 w7c, 16:1w6c and/or 15:0 iso 2OH; summed feature 7: one or more among unknown 18.846 and/or 19:1 w6c; summed feature 8: one or more among 18:1 w7c and/or 18:1 w6c.